

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB NO. 0704-0188

Public Reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comment regarding this burden estimates or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188,) Washington, DC 20503.

1. AGENCY USE ONLY (Leave Blank)

2. REPORT DATE  
October 1, 2001

3. REPORT TYPE AND DATES COVERED  
Final Progress Report (4/1/98 - 3/31/01)

4. TITLE AND SUBTITLE  
Uncovering the Genetic Basis of Sleep: Use of Clock Mutant Mice

5. FUNDING NUMBERS

DAA655-98-1-0196

6. AUTHOR(S)  
Fred W. Turek, Ph.D., and Martha H. Vitaterna, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  
Northwestern University  
Rebecca Crown Hall, 633 Clark Street  
Evanston, IL 60208

8. PERFORMING ORGANIZATION  
REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U. S. Army Research Office  
P.O. Box 12211  
Research Triangle Park, NC 27709-2211

10. SPONSORING / MONITORING  
AGENCY REPORT NUMBER

38532.3-LS

11. SUPPLEMENTARY NOTES

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

12 a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

12 b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Our overall goal is to use the mouse to identify genetic elements controlling sleep. We will use the *Clock* mutant mouse because of finding that *Clock* mutants, in addition to their circadian abnormalities, sleep less than wild-type mice.

Over the past year, we have examined how the *Clock* mutation alters sleep and body temperature rhythms under stressful conditions. Correspondingly, body temperature decreased during the recovery dark period in wild-types but did not change in *Clock/Clock* mice. Finally, a negative rebound in delta power was observed in wild-type mice, but not in *Clock/Clock* mice. These results indicate that the *Clock* gene disrupts the pattern and amount of sleep, as well as the amplitude of the body temperature rhythm under baseline and stressful conditions.

We have also examined sleep in neonatal pups to determine if pups could be used in mutagenesis screening. Our findings in mice, and in other studies in rats, that a simple behavior in the neonate can be used to detect genetic differences observed in adult sleep represent a "proof of principle" that it will be possible to use behavioral states in the neonate to uncover unknown genes involved in the regulation of the sleep-wake cycle of mammals.

Other studies carried out under this proposal are described in the next page.

14. SUBJECT TERMS  
Sleep, Genetics, Circadian Rhythms, Mice

15. NUMBER OF PAGES

3

16. PRICE CODE

17. SECURITY CLASSIFICATION  
OR REPORT  
UNCLASSIFIED

18. SECURITY CLASSIFICATION  
ON THIS PAGE  
UNCLASSIFIED

19. SECURITY CLASSIFICATION  
OF ABSTRACT  
UNCLASSIFIED

20. LIMITATION OF ABSTRACT  
UL

NSN 7540-01-280-5500

Standard Form 298 (Rev.2-89)  
Prescribed by ANSI Std. Z39-18  
298-102

20011101 113

---

**REPORT DOCUMENTATION PAGE (SF298)**  
**(Continuation Sheet)**

---

**1. List of Manuscripts**

Dugovic C, Solberg LC, Redei E, Van Reeth O and Turek FW. Sleep in the Wistar-Kyoto rat, a putative genetic animal model for depression. *NeuroReport* 2(3):627-631 (2000).

Pinto L, Vitaterna MH and Turek FW. Section Four – Molecular Genetic Basis for Mammalian Circadian Rhythms. In: *Principles and Practice of Sleep Medicine, 3rd Edition* (MH Kryger, T Roth and WC Dement, eds.). WB Saunders, New York, pp 346-352 (2000).

Naylor E, Bergmann BM., Krauski K, Zee PC., Takahashi JS., Vitaterna MH, and Turek FW. The circadian *Clock* mutation alters sleep homeostasis in the mouse. *J. Neuroscience* 20(21):8138:8143 (2000).

Reid KJ, Chang, AM, Dubocovich ML, Turek, FW, Takahashi, JS and Zee, PC. Familial Advanced Sleep Phase Syndrome. *Archives of Neurology*, 50(7):1089-1094, (2001).

**Abstracts:**

Battle S, Dugovic C and Turek FW. Similar genetic control of neonatal and adult sleep in mice. *Soc. Res. Biol. Rhythms*, Abstract. 190, page 117 (2000).

Easton A, Bergmann B and Turek FW. Clock mutant mice show altered sleep and body temperature rhythms under baseline and stressful conditions. *Soc. Res. Biol. Rhythms*, Abstract. 277, page 161 (2000).

Cronin SJ, Nasim A, Naylor E, Takahashi JS and Turek FW. Sleep deprivation in the clock mutant mouse. *Associated Professional Sleep Societies*, Abstract 1742.E, page A186, Vol 23(Abs Sup #2) (2000).

Turek FW. Dissecting complex behaviors with molecular genetics: Lessons from clock genes for sleep genes. *XXIInd CIMP Abstracts*, Brussels, Belgium, 2000.

Turek FW, Dugovic C and Battle S. Development of sleep in mice with different genetic backgrounds and with altered sleep phenotypes in adults. Presented at the 15<sup>th</sup> Congress of the European Sleep Research Society. *J. Sleep Res* 9(1):194, Abstract 387, (2000).

Easton A, Koehl M, Turek FW, Meerlo P. Time of day effects of stress on sleep in C57BI/6J mice. *Sleep*, 24:324.E (2001).

Meerlo P, Bergmann BM, Turek FW. Restraint Stress Induces a Pronounced Increase in Prolactin and a Specific Increase in Rapid Eye Movement (REM) Sleep in C57BL/6 Mice but not in BALB/c Mice. The 15<sup>th</sup> annual meeting of the Sleep Research Society, Chicago, June 2001. *Sleep*, Vol. 24, Abstract Supplement, (2001).

**2. Scientific personnel**

P.I.: Fred W. Turek was supported in part by this grant.

Ph.D. Students: Supported by this grant was Mr. Erik Naylor, while other students worked on this project, but were supported by other grants, including Ms. Amy Easton and Ms. Sara Cronin.

Research Technologists: Supported by this grant included Ms. Sally Battle, Ms. Susan Olsen, Mr. William Olsen and Ms. Julie Uherka.

Postdoctoral Fellows/Research Associates: Supported by this grant included Dr. Martha Vitaterna and Dr. Muriel Koehl.

**3. Report of Inventions**

None.

**4. Scientific Progress and Accomplishments. The results of three separate sets of studies add to our previous progress reports. These include:**

1. Clock Mutant Mice Show Altered Sleep and Body Temperature Rhythms Under Baseline and Stressful Conditions.

The circadian pacemaker is known to regulate the timing and distribution of sleep; however, the role of specific circadian genes in the regulation of sleep has just begun to be explored. The first mammalian circadian clock gene to be discovered, *Clock*, is highly expressed in the eye and suprachiasmatic nuclei and is an integral part of the pacemaker's molecular machinery. Mice bred with a mutation of the *Clock* gene (*Clock/Clock*) exhibit significantly altered free-running rhythm in running wheel activity. In this study, we were specifically interested in determining whether recovery

sleep following an acute stress was affected by the *Clock* gene mutation. Six wild-type and 8 *Clock/Clock* male mice bred on a C57/B1/6J background and maintained on a L:D 12:12 cycle. Mice were implanted with EEG and EMG electrodes and a Minimitter transmitter to collect data on body temperature and total locomotor activity. Baseline sleep was collected for 24 hours at least 1 week after recovery from surgery. On day 2, animals were restrained in a plastic tube for 1 hour between ZT3-5. Recovery sleep was recorded for the following 24 hour period immediately after the stress ended. *Clock/Clock* mice showed a decrease in NREM sleep ( $p<.001, t=4.04$ ) during the light period and twice as much REM sleep during the dark period ( $p<.001, t=3.6$ ) during baseline recording. In addition, *Clock/Clock* mice had less delta power during the dark period compared to wild-types and the amplitude of the body temperature rhythm was decreased in *Clock/Clock* mice, which corresponded with the changes in NREM and REM sleep across the light/dark cycle ( $p<.05, f=7.8$ ). Following an acute stressor, *Clock/Clock* mice showed less recovery sleep than wild-type mice ( $p<.05$ ). Correspondingly, body temperature decreased during the recovery dark period in wild-types but did not change in *Clock/Clock* mice. Finally, a negative rebound in delta power was observed in wild-type mice, but not in *Clock/Clock* mice ( $p<.01$ ). These results indicate that the *Clock* gene disrupts the pattern and amount of sleep as well as the amplitude of the body temperature rhythm under baseline and stressful conditions.

## 2. In Mice Restraint Stress at Light Onset Produces an Increase in REM Sleep Whereas Restraint at Dark Onset Does Not.

**Introduction:** Restraint stress induces a specific increase in REM sleep in rodents. However, whereas most physiological, endocrine and behavioral consequences of restraint are strongly dependent on the time of day at which it takes place no study has directly addressed the question whether the REM sleep promoting effect of restraint stress is also time dependent.

**Methods:** Adult male C57BL/6J mice were implanted with permanent electrodes to record EEG and EMG. After at least 2 weeks of recovery, sleep-wakefulness patterns were recorded for 2 days. The first day was a baseline recording and on the second day the mice were subjected to 1h of restraint stress starting at either the onset of the light phase ( $n=8$ ) or at the onset of the dark phase ( $n=8$ ).

**Results:** Restraint stress at light onset resulted in a significant increase in REM sleep, especially in the subsequent dark phase. This increase in REM sleep was much larger than the amount of REM sleep that was lost during restraint and therefore could not be attributed to a rebound from sleep deprivation. In contrast, restraint stress at dark onset did not induce a significant increase in REM sleep, neither in the remainder of the dark phase nor in the subsequent light phase.

**Conclusions:** These results support earlier findings that restraint stress can induce an increase in REM sleep in mice. At the same time, however, the data show that this REM sleep inducing effect is strongly dependent on the time of day at which the restraint takes place.

## 3. Development of Sleep in Mice with Different Genetic Backgrounds and with Altered Sleep Phenotypes in Adults.

Understanding the relationship between sleep-wake states in neonates and adults is critical for any complete understanding of not only the ontogeny of sleep, but also for understanding the regulatory mechanisms underlying the transitions between states of vigilance, as well as for addressing critical questions concerning the function(s) or need for sleep. At the present time, there is considerable controversy as to the relationship between the active sleep (AS) and quiet sleep (QS) states in neonates and the EEG defined states of REM and NREM sleep in adults. Clear genetic differences in the characteristics of the behavioral sleep states in adult animals of different strains of rodents offer a potential window for examining, at least at the genetic level, how the neonatal and adult behavioral states are related to one another.

For this purpose, short-lasting recordings (3-h period during the light phase) of behavioral states of vigilance were performed in neonatal (8-day old) mice of four different strains (C57BL/6ByJ, BALB/cByJ, DBA/2J and C57BL/6J) which show different amounts of vigilance states as adults. Many of these differences were already evident when wake, QS and AS were monitored only via EMG recordings in 8-day old mice. Interestingly, despite the absence of circadian sleep-wake rhythms in neonates, it was also possible to detect sleep-wake differences between strains that appeared to be restricted later in life to the light or the dark phase in adult animals.

The present results indicate that behavioral sleep states in the neonate are under similar genetic control as the REMS and NREMS states in adult animals. While it is not known how neonatal AS and QS are physiologically related to adult REM and NREM sleep, respectively, the finding of similar differences in the amounts of sleep-wake states in neonatal and adult life argues strongly that at some level they are controlled by similar cellular/physiological mechanisms. Furthermore, the fact that these differences between strains of mice can be detected even before EEG defined sleep-wake states can be differentiated, indicates that the neonate can be used to screen for genetic abnormalities of sleep-wake states in a large number of animals. Such a screen is vital in order to use a forward genetics approach to elucidate the genetic

basis for sleep and wake states. Our findings in mice, and in other studies in rats, that a simple behavior in the neonate can be used to detect genetic differences observed in adult sleep represent a "proof of principle" that it will be possible to use behavioral states in the neonate to uncover unknown genes involved in the regulation of the sleep-wake cycle of mammals.

**5. Technology transfer**

Not applicable.

**MASTER COPY:** PLEASE KEEP THIS "MEMORANDUM OF TRANSMITTAL" BLANK FOR REPRODUCTION PURPOSES. WHEN REPORTS ARE GENERATED UNDER THE ARO'S SPONSORSHIP, FORWARD A COMPLETED COPY OF THIS FORM WITH EACH REPORT SHIPMENT TO THE ARO. THIS WILL ASSURE PROPER IDENTIFICATION. NOT TO BE USED FOR INTERIM PROGRESS REPORTS; SEE PAGE 2 FOR INTERIM PROGRESS REPORT INSTRUCTIONS.

**MEMORANDUM OF TRANSMITTAL**

U.S. Army Research Office  
ATTN: AMSRL-RO-BI (TR)  
P.O. Box 12211  
Research Triangle Park, NC 27709-2211

☐ Reprint (Orig + 2 copies)

☐ Technical Report (Orig + 2 copies)

☐ Manuscript (1 copy)

☒ Final Progress Report (Orig + 2 copies)

☐ Related Materials, Abstracts, Theses (1 copy)

CONTRACT/GRANT NUMBER:

REPORT TITLE:

is forwarded for your information.

SUBMITTED FOR PUBLICATION TO (applicable only if report is manuscript):

Sincerely,

*Fred W. Turek*